AN ANALYSIS OF THE STAGES OF NEURULATION IN THE COMMON AMERICAN FROG (Rana pipiens) BY MEANS OF THE MITOTIC INDEX

BY
GORDEN WILBUR HASSE
A.B., Oberlin College, 1937
M.A., Oberlin College, 1939

AN ABSTRACT OF A THESIS
SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
ZOOLOGY IN THE GRADUATE SCHOOL OF THE
UNIVERSITY OF ILLINOIS, 1942

URBANA, ILLINOIS
1942

AN ANALYSIS OF THE STAGES OF NEURULATION IN THE COMMON AMERICAN FROG (Rana pipiens) BY MEANS OF THE MITOTIC INDEX

BY
GORDEN WILBUR HASSE
A.B., Oberlin College, 1937
M.A., Oberlin College, 1939

AN ABSTRACT OF A THESIS
SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
ZOOLOGY IN THE GRADUATE SCHOOL OF THE
UNIVERSITY OF ILLINOIS, 1942

URBANA, ILLINOIS
1942

ACKNOWLEDGMENTS

I wish to express my grateful appreciation to Professor Waldo Shumway under whose guidance this investigation was undertaken and to Professor F. B. Adamstone whose criticism and helpful suggestions have been invaluable throughout this study.

AN ANALYSIS OF THE STAGES OF NEURULATION IN THE COMMON AMERICAN FROG (Rana pipiens) BY MEANS OF THE MITOTIC INDEX

Since the beginning of the present century a great volume of experimental work has been done on the embryology of the Amphibia, and purely morphological studies have been lacking to a large extent. In the last ten or fifteen years, however, there has appeared a sufficient amount of morphological research to suggest a renewal of a more precise search for facts concerning normal development in an effort to strengthen and expand existing theories of the processes involved in gastrulation and organ formation. The present work on the analysis of neurulation stages of *Rana pipiens* by means of the mitotic index was undertaken in an effort to add to existing knowledge of these stages by utilizing a relatively unused technique, for it is only through the compilation of the facts obtained by all available techniques that we will be able to reconstruct the whole story of development.

Materials and Methods

The embryos used in this analysis were obtained either by means of artificially induced ovulation and insemination or from local pools where *Rana pipiens* was known to breed. The embryos were fixed in Smith's frog fluid for 24 hours, washed thoroughly in 5% formalin, and the jelly removed by shaking in a 10% solution of Chlorox. The embryos were dehydrated through graded alcohols, cleared in benzene, and embedded in a mixture of two parts 52° paraffin and one part Parawax (Standard Oil Co.). Transverse serial sections were cut at twenty micra.

To ensure against loss of sections, each slide was given a thin coating of celloidin before staining with Feulgen's stain. The slides were hydrolyzed forty-five minutes and stained approximately two hours. After thorough washing in sulfurous acid water the sections were counter-stained

in picro-indigo-carmine, fast green, or a mixture of these two stains, dehydrated, cleared in xylene, and mounted in clarite.

The embryos used in this study were carefully examined, both from the exterior and from the serial cross-sections, to ensure the use of only normal individuals in the tabulation of the data on the mitotic indices.

Since in this study it was desired to examine every region of each embryo in order to get as complete a record of the mitotic activity as possible, only those cells in unquestionable stages of mitosis were counted. The stages used were: 1) very late prophase, 2) metaphase, 3) anaphase, and 4) early telophase, and the few cells which were difficult to distinguish were considered as non-dividing cells. By using just these stages it was possible to tabulate data on alternate sections of each embryo. Of course this method gives lower indices than are actually the case, but as long as the criteria used in the counting remain constant the indices obtained will be comparable, and for this study that is sufficient.

Cell counts were made at a magnification of approximately 450X with a Leitz binocular compound microscope equipped with a mechanical stage. An ocular provided with cross-hairs was used to prevent recounting cells in the section. Different types of cells or different tissues of the embryo were tabulated separately as far as was consistent with accuracy.

Periodically during the tabulation of the data sections chosen at random were recounted to determine the constancy of observation. In all these recounts the number of mitotic figures checked exactly with the original data. The counts of the non-dividing cells varied slightly in some cases but not enough to affect the index significantly.

The mitotic indices were then computed to the nearest one hundredth of one percent for all the different parts of each section and graphs prepared on a moving average of three to facilitate comparison of various tissues as well as the different stages of each tissue. Graphic reconstructions

in the median sagittal plane were made of the different embryos in order to compare the different stages.

Observations

In the embryo of early stage 13 the major part of the mitotic activity is in the posterior part of the embryo.

In the late stage 13 there are three regions of higher mitotic index in the nervous ectoderm: the first in the region of the presumptive brain; the second in the region of the anterior third of the presumptive neural tube; and the third in about the middle of the presumptive tube.

The general pattern of the mitotic activity in this stage is similar to that of the earlier stage but at a higher level. There is also a secondary peak of activity at the anterior end.

The chorda-mesoderm shows a general increase in activity with the addition of a high point at the extreme posterior end of the embryo.

In stage 14 the nervous ectoderm exhibits an extensive region of higher index (usually above 3.0) while the posterior two-thirds averages only about 1.0.

The medullary plate has three definite peaks of mitotic activity each of which decreases posteriorly. The first is at the anterior end, the second is in the region of the anterior end of the future neural tube, and the third is about the middle of the future tube region.

The sense plate appears as a region of high index in the anterior end of the embryo, while the gill plate shows a fall in rate of mitosis to about half that of the sense plate.

There is a broad region of high index in the endoderm just anterior to the liver and a region of low index in the vicinity of the liver. There is a small region of high activity at the extreme posterior end of the endoderm.

The mesoderm, including the mesendoderm in the anterior shows high rates of mitosis at the anterior and posterior ends of the embryo with a low rate in the middle.

In the nervous ectoderm of stage 15 the peak about one-fourth the way posterior corresponds very closely with the region in which the otic vesicles are visible in the following stage. There is a slight secondary peak in the posterior

end of the embryo.

In the medullary plate of this stage there is a slight increase from the anterior end to the posterior end of the future brain. The neural tube region exhibits an irregular increase in rate in the middle decreasing anteriorly and posteriorly.

The posterior part of the gill plate has a local region of high mitotic index and the anterior region of the gill plate and the sense plate have about equal activity. This contrasts with the previous stage in which the conditions were just the reverse.

The endoderm has a local region of high mitotic index in the anterior end which falls off rapidly and is followed by another peak which decreases rapidly to the middle third of the embryo. There is a slight increase in the index toward the posterior end.

The mesoderm has a local region of activity at the anterior end decreasing to the posterior third of the embryo. There is a secondary increase in the posterior third.

In stage 16 the nervous ectoderm has become decidedly more active than in the previous stage, especially in the middle region.

In the neural tube there is a decrease in the index posteriorly through the brain and anterior cord region. This is in contrast to the situation observed in stage 15. The peak in the middle region of the cord has become narrower, but there is in addition a lower plateau farther posterior.

The sense plate as a whole is slightly more active than in the previous stage and shows a rather definite increase in the posterior half. The mid-region of the gill plate exhibits a very definite peak in activity which decreases rapidly both anteriorly and posteriorly.

The endoderm of stage 16 is characterized by a broad plateau in the anterior region and a small peak in the posterior region.

There is a rather sharp increase in the index from the anterior end of the mesoderm posteriorly to the region of the anterior end of the neural tube. The graph of the in-

dices of the middle region of this tissue shows a gradual decrease in rate of mitosis with periodic dips, finally ending in a relatively small region of increased mitosis.

The pronephric tissue present at this stage presents essentially a bi-modal curve.

In stage 17 the nervous ectoderm shows three definite peaks in mitotic activity: the first in the region of the hind-brain, the second about the region of the middle of the neural tube, and the third at the posterior end of the embryo.

The anterior half of the central nervous system exhibits a rather high mitotic index, with a broad peak in the anterior region of the neural tube. Posteriorly there is a general decrease with a subsequent rise toward the posterior end.

The gill plate has a slightly lower and more uniform index than was observed in the previous stage.

The mitotic index of the endoderm is about twice that of the previous stage. The general pattern is the same but at a higher level.

The mesoderm shows a slight decrease in mitotic index from the anterior end toward the middle of the embryo. There is a definite increase from the middle region to a peak of activity almost at the posterior end of the mesoderm.

The pronephros still presents essentially a bi-modal curve, but the peaks of activity are about three times as high and the index of the tissue as a whole is double that of the previous stage.

Summary and Conclusions

As a result of the studies of the mitotic indices of *Rana pipiens* the following conclusions appear to be warranted:

1) The areas of greatest mitotic activity in any tissue of an embryo are not necessarily arranged in a directional sequence at any particular stage of development. Moreover they may shift in position with age and development of the embryo, and no region of a given tissue has a constant high rate of mitosis.

2) In any embryo not all tissues at a particular level show equally high mitotic activity. Areas of high mitotic activity in one or more embryonic tissues are often associated with areas of relatively low activity in other tissues at the same levels.

3) Areas of mitotic activity at any given level may shift or alternate from one embryonic tissue to another with age or degree of development.

4) The results of these studies amply demonstrate that periods of mitotic activity precede actual differentiation of the various primordia. As a result these can be identified in sections before they are externally visible. A striking example of this point is found in the demarcation of the region of the future otic vesicle in stage 15 before it is visible in stage 16.

VITA

Gorden Wilbur Hasse was born in Cleveland, Ohio, January 28, 1916. He attended a Cuyahoga elementary school and two and one-half years of high school. He was graduated from James Ford Rhodes High School, Cleveland, Ohio, in June, 1933.

In September, 1933, he entered Oberlin College, Oberlin, Ohio, and received the degree of Bachelor of Arts in 1937 and the degree of Master of Arts in 1939 from that school. He assisted in the zoology laboratories at Oberlin College from 1937 to 1939.

From September, 1939, to September, 1942, he was a teaching assistant in the Department of Zoology of the University of Illinois. The summer of 1941 was spent in study at the Marine Biological Laboratory, Woods Hole, Massachusetts.

He is a member of Gamma Alpha and Phi Sigma and an Associate Member of Sigma Xi.